$G\alpha_q$ PROTEIN VARIANTS AND THEIR USE IN THE ANALYSIS AND DISCOVERY OF AGONISTS AND ANTAGONISTS OF CHEMOSENSORY RECEPTORS

Cross-Reference to Related Applications

[0001] This application is a continuation-in-part of U.S. Application Serial No. 09/984,292 filed October 29, 2001, which claims priority to U.S. Provisional Application No. 60/243,770, filed on October 30, 2000, and are incorporated herein by reference in their entirety.

Field of Invention

[0002] This invention relates to Gαq protein variants and their use in the analysis and discovery of agonists and antagonsists of chemosensory receptors, such as G protein coupled receptors involved in sensing of tastants, olfactants, and pheromones.

Background of Invention

[0003] Heterotrimeric G proteins, consisting of alpha, beta and gamma subunits, couple ligand-bound seven transmembrane domain receptors (GPCRs or G-protein coupled receptors) to the regulation of effector proteins and production of intracellular second messengers such as cAMP, cGMP, and Ca^{2+}. G protein signaling mediates the perception of environmental cues in all higher eukaryotic organisms, including yeast, Dictyostelium, plants, and animals. Agonist-bound sensory receptors catalyze the exchange of GTP for GDP on the surface of the Gα subunit to initiate intracellular responses to extracellular signals. Intracellular signaling is mediated through various effector enzymes, including cGMP phosphodiesterase, phospholipase C, adenylate cyclase, etc. (see Kinnamon & Margolskee, 1996, Curr. Opinion Neurobiol. 6: 506-513). Most effector proteins interact with the Gα, although Gβγ subunits also contribute to the specificity of receptor-G protein coupling (Xu et al., 1998, J. Biol. Chem. 273(42): 27275-79).

[0004] The G protein α subunits are grouped into four families, $G\alpha_s$, $G\alpha_i$, $G\alpha_q$, and $G\alpha_{12}$ according to their sequence homologies and functional

similarities. The $G\alpha_q$ family members couple a large group of GPCRs to phospholipase C. Activation of $G\alpha_q$ coupled GPCRs induces intracellular calcium release and the capacitative calcium entry from extracellular space. The consequential increase of cytosolic calcium concentration can be effectively detected by using synthetic or genetically-engineered fluorescent calcium indicators, bioluminescent calcium indicators, calcium-activated ion currents, and by monitoring calcium-regulated gene transcription. Assays based on such calcium readout are available in high-throughput screening (HTS) format.

[0005] Signaling specificity among α subunits of the same class having similar biochemical functions is not well understood *in vivo*. For instance, the $G\alpha_q$ (G_q) class includes four proteins expressed in mammals, called $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14}$, and $G\alpha_{15}$ (in mice, $G\alpha_{16}$ in humans). Whereas orthologs of these subunits are highly conserved across species (99, 97, 96 and 85% identity, respectively), paralogs of these subunits (expressed in the same species) are not as conserved. This suggests that each type of subunit in the G_q class has a distinct function, however, when transfected into Sf9 cells, the subunits stimulated phospholipase C with similar potency and showed similar activities (Nakamura et al., 1995, J. Biol. Chem. 270: 6246-6253). Xu and colleagues subsequently showed by gene knockouts in mice that Gq_α subunits promiscuously couple to several different receptors in various cell types (1998, J. Biol. Chem. 273(42): 27275).

[0006] The promiscuity of the $G\alpha_q$ subclass of G protein subunits provides a valuable tool for analyzing the role of G protein complexes and GPCRs in chemosensory transduction. For instance, the ability of $G\alpha_q$ proteins to bypass the selectivity of the receptor G-protein interaction can be used to study the molecular mechanism of receptor-induced G-protein activation. In addition, the promiscuity toward receptors may be helpful in identifying ligands corresponding to orphan receptors whose signaling properties are unknown. Promiscuous G protein subunits play a particularly useful role in generating screening assays for high affinity GPCR agonists, antagonists, and modulators of chemosensory activity, in that using a single G protein coupler removes the

variability of the G protein from the equation, thereby simplifying interpretation of results gleaned from various modulating compounds and GPCRs. Chemosensory modulating compounds involved in taste and/or smell, for instance, could then be used in the pharmaceutical and food industries to customize taste or aroma. In addition, such chemosensory molecules could be used to generate topographic maps that elucidate the relationship between the taste cells of the tongue or olfactory receptors (Ors) and sensory neurons leading to the brain.

[0007] Despite their promiscuity, however, $G\alpha_q$ class subunits do not mediate all GPCR – effector interactions. For instance, human $G\alpha_{16}$ and its murine counterpart $G\alpha_{15}$ are promiscuous G proteins in that they couple to GPCRs of different G protein families (Offermanns and Simon, 1995; Negulescu et al., 1997). However, they are not true universal adapters for GPCRs in that there are at least 11 GPCRs reported to be incapable of activating Gα15/Gαl6 (Wu et al., 1992; Arai et al., 1996; Kuang et al., 1996; Lee et al., 1998; Parmentier et al., 1998; Mody et al., 2000). Similar problems arise when using $G\alpha_{15}/\alpha_{16}$ to identify ligands of ORs and T2Rs (bitter taste receptors) in that (1) calcium responses to odorants are small and quickly desensitized for ORs in $G\alpha_{15}/\alpha_{16}$ transiently transfected cells (Krautwurst et al., 1998); (2) most T2Rs remain orphan using cell lines stably transfected with $G\alpha_{15}$ (Adler et al., 2000; Chandrashekar et al., 2000); and (3) threshold concentration of denatonium measured is at least one order higher than expected for bitter receptors, hT2R4 and mT2R8 expressed in cells stably transfected with $G\alpha_{15}$ (Adler et al., 2000; Chandrashekar et al., 2000). These problems suggest that the coupling efficiency between ORs/T2Rs and $G\alpha_{15}/\alpha_{16}$ is weak and may vary within the family of ORs and T2Rs.

[0008] Given the partial promiscuity of $G\alpha_q$ class proteins, it would be desirable to identify or create Gα protein subunits that are more promiscuous than their native counterparts, and which are capable of interacting with a wider variety GPCRs.

Summary of Invention

[0009] The present invention addresses the above described problems associated with using $G\alpha_{15}/\alpha_{16}$, as well as other problems known in the art relating to the use of weakly promiscuous $G\alpha$ proteins. Generally, the invention provides a series of $G\alpha_q$ (G_q class) protein variants that functionally couple sensory cell receptors such as taste GPCRs (TRs) and olfactory GPCRs (ORs). According to the invention, the functional coupling can be determined, for example, by measuring changes in intracellular IP3 or calcium. In a particular embodiment, the G_q protein variants can be expressed in mammalian cell lines or *Xenopus* oocytes, and then evaluated using calcium fluorescence imaging and electrophysiological recording.

[0010] In one aspect of the invention, G alpha class q (G_q) variants that are capable of widely promiscuous functional coupling to chemosensory receptors, such as taste and olfactory receptors, and isolated nucleic acid sequences encoding the same are provided. Another aspect of the invention is directed to chimeric G_q variants and the isolated nucleic acids encoding the same. In one embodiment, the chimeric G_q protein variants comprise C-terminal sequences from transducin or $G\alpha_{olf}$, which exhibit improved functional coupling to taste and olfactory receptors, respectively.

[0011] In yet another aspect of the invention, a method for the analysis and discovery of agonists and/or antagonists of chemosensory receptors using the G_q protein variants is provided. One embodiment is directed to a mammalian cell-based assay using a transiently transfected gene or cDNA encoding a G_q protein variant. Another embodiment is directed to a mammalian cell-based assay using a stably expressed gene or cDNAs encoding G_q protein variants. In yet another embodiment, a method for analysis and discovery of agonists and/or antagonists of chemosensory receptors in *Xenopus* oocytes using genes, RNAs or DNAs encoding G_q protein variants is provided. The agonists and/or antagonists discovered using the disclosed assays are also encompassed, as are antibodies which bind specifically to the G_q variants described herein, but not those which also bind to known G_q proteins.

[0012] Other aspects of the invention relate to expression vectors comprising nucleic acid sequences encoding the G_q protein variants of the invention, as well as host cells comprising such expression vectors. Further aspects of the invention will become apparent to one of skill in the art from the following detailed description and examples.

Brief Description of the Figures

[0013] Figure 1 illustrates the alignment of amino acid sequences of human $G\alpha_q$, $G\alpha_s$ and $G\alpha_{16}$ by the Clustal method.

[0014] Figure 2 illustrates the amino acid sequences of mouse and human $G\alpha_q$. Significant amino acids described herein are boxed and differences between human and mouse are underlined.

[0015] Figure 3 illustrates the amino acid sequences of mouse and human $G\alpha_q$ proteins according to the invention. The variations of the amino acids of $G\alpha_q$ are depicted in parenthesis. The sequence numbers of amino acid H or Q, V or L are 28 and 29 respectively. The sequence number of amino acid G or D is 66. Truncation of N-terminal six amino acids (MTLESI) are shown as ΔN. Hemaglutinin (HA) epitope tag (DVPDYA) spans from 125 to 130. C-terminal five amino acids (-t5) or 44 amino acids (-t44) of transducin and five amino acids of $G\alpha_{olf}$ (-olf5) are used respectively to replace those of $G\alpha_q$.

[0016] Figure 4 illustrates additional amino acid sequences according to the invention.

Detailed Description of the Invention

[0017] As described above, there are known problems with the use of $G\alpha_{15}/G\alpha_{16}$ to couple chemosensory receptors in that the coupling efficiency between ORs/T2Rs and $G\alpha_{15}/\alpha_{16}$ is weak and may vary within the family of ORs and T2Rs (bitter taste receptors). As such, the invention provides a series of G_q protein variants that functionally couple sensory cell receptors such as taste GPCRs (TRs) and olfactory GPCRs (ORs) in a promiscuous manner. According to the invention, "promiscuous" or "promiscuity" refers to the ability to functionally couple to more than one taste GCPRs and/or olfactory GPCRs.

"Increased promiscuity" or "widely" promiscuous refers to the ability to functionally couple to more taste GCPRs and/or olfactory GPCRs than would be demonstrated by the native G_q protein.

[0018] The term "G_q" as used herein encompasses all the $G\alpha_q$ subclasses, including $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14}$, and $G\alpha_{15}$ (in mice, $G\alpha_{16}$ in humans). However, the chimeric promiscuous or widely promiscuous G_q proteins described herein may have sequences incorporated from other $G\alpha$ class proteins, for instance, from $G\alpha_s$, $G\alpha_i$ and $G\alpha_{12}$. The existing variation between members of the G_q class could be utilized in combination with the characteristic of promiscuity to generate promiscuous G_q proteins having altered or new receptor specificities. Protein sequence similarity between $G\alpha_q$ and $G\alpha15$/ $G\alpha_q16$ is less than 57% (Fig. 1). Accordingly, such high divergence should result in significant differences in efficiency and selectivity of receptor coupling. The identification of functionally active Gq protein variants could allow for the pharmacological and genetic modulation of sensory transduction pathways.

[0019] For example, Gq protein variants could enable screening for high affinity agonists, antagonists, inverse agonists, and other modulators of sensory cell transduction and activity. Such sensory cell modulators could then be used in the pharmaceutical and food industries to customize sensory perceptions. In addition Gq protein variants could serve as tools in the generation of sensory topographic maps.

[0020] According to the invention, Gq protein variants include variants having point mutations that increase promiscuity with regard to GPCR coupling. For instance, the inventors have found that mouse $G\alpha_q$ variants comprising a Gly to Asp change at position 66 (G66D) demonstrate increased promiscuity. Similar mutations are predicted to have a similar effect on the activity of the corresponding human $G\alpha_{16}$ subunit given the level of homology and similar activity demonstrated between the two proteins. The mutation G66D is localized at linker 1 region between helices $\alpha1$ and αA of $G\alpha_q$ (Lambright et al., 1996). For reference, the amino acid sequences for mouse and human $G\alpha_q$ are listed in Figure 2.

[0021] It was found by functional analysis using single-cell calcium imaging that activation of multi-family GPCRs evoked increases in cytosolic calcium in the presence of the $G\alpha_q$ variants with the G66D mutation. These GPCRs include $G\alpha_s$-coupled β-adrenergic receptor, $G\alpha_{olf}$-coupled mouse I7 olfactory receptor, $G\alpha_i$-coupled m2 muscarinic receptor, and gustducin-coupled bitter receptor mT2R5. No significant change in cytosolic calcium could be detected by activation of the above GPCRs in the absence of the $G\alpha_q$ variants. Additional GPCRs can include those disclosed in U.S. Patent Application No. 09/510,332 filed February 22, 2000; and U.S. Provisional Application Nos. 60/213,849 filed June 23, 2000; 60/209,840 filed June 6, 2000; 60/195,536 filed April 7, 2000; 60/195,534 filed April 7, 2000; 60/195,532 filed April 7, 2000; which are herein incorporated by references for all purposes in a manner consistent with this disclosure.

[0022] Thus, $G\alpha_q$ variants according to the invention can comprise amino acid substitutions at or near position 66, or at any other position that results in an increase of promiscuity by the variant Gα protein. Other G_q subclass variants can be designed having similar mutations. Mutations can be identified and isolated using site directed or random mutagenesis according to techniques that are known in the art, including random saturation mutagenesis around the mutation sites described herein. The variants may comprise these one or more of these mutations alone or in combination with C-terminal substitutions. In another embodiment of the invention, $G\alpha_q$ and other G_q subclass variants comprise C-terminal sequences derived from other G proteins.

[0023] For instance, the present inventors have also discovered that the Gly to Asp mutation is synergistic with the replacement of the C-terminus of $G\alpha_q$ by that of transducin or $G\alpha_{olf}$. $G\alpha_q$ proteins containing C-terminal amino acids from transducin or $G\alpha_{olf}$ in combination with a Gly66 to Asp alteration show increased activity compared to individual chimeras alone. A preferred embodiment is a variant G_q proteins having at least about five amino acids in the C terminus of said G_q protein replaced by at least about five amino acids

from the C terminus of $G\alpha_{olf}$ or transducin, wherein said C-terminal substitution increases promiscuity of said variant G_q protein as compared to the corresponding native G_q protein. Up to 44 amino acids of the C terminus of transducin or $G\alpha_{olf}$ may be incorporated. Other possible variants are shown in Figures 3 and 4.

[0024] Other mutations and substitutions are envisioned to be within the scope of the invention. For instance, it would be within the level of skill in the art to perform additional amino acid substitutions at other amino acid positions using known protocols of recombinant gene technology including PCR, gene cloning, site-directed mutagenesis of cDNA, transfection of host cells, and in-vitro transcription. The variants could then be screened for functional coupling to chemosensory receptors as described herein. Further, additional C-terminal substitutions could be made from other G-protein molecules known in the art.

[0025] The present invention also includes isolated $G\alpha_q$ subunit polypeptide variants comprising polypeptides with greater than 80% amino acid sequence identity to a sequence selected from the group consisting of SEQ ID Nos 1-26. More preferably, variants comprising polypeptides with greater than 90% amino acid sequence identity are included, with the most preferable homologs being at least about 95% identical to the variants described herein.

[0026] The invention also includes isolated nucleic acid sequences encoding the G_q protein variant polypeptides of the invention. Included are isolated nucleic acid sequences comprising a nucleic acid encoding a polypeptide with greater than 80% amino acid sequence identity to a sequence selected from the group consisting of SEQ ID Nos 1-26. More preferably, the isolated nucleic acid sequence encoding a $G\alpha_q$ protein variant comprises a nucleic acid encoding a polypeptide with greater than 90% amino acid sequence identity to a sequence selected from the group consisting of SEQ ID Nos 1-26. Most preferred are isolated nucleic acid sequences encoding $G\alpha_q$ protein variants which comprise a nucleic acid encoding a polypeptide with greater than about 95% amino acid sequence identity to a sequence selected from the group consisting of SEQ ID Nos 1-26.

[0027] The terms "identical" or "percent identity" in the context of two or more protein or nucleic acid sequences refers to sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same when compared and aligned for maximum correspondence over a comparison window or designated region, using either a sequence comparison algorithm that is known in the art or by manual inspection. Sequences with over 80% sequence identity are said to be "substantially identical." Optionally, the identity, exists over a region that is at least about 25-30 amino acids or nucleotides in length, or optionally over a region that is 75-100 amino acids or nucleotides in length.

[0028] For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. A "comparison window" as used herein includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 25 to 500, usually about 50 to about 200, more usually about 100 to 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences are well known in the art (see, e.g., Smith and Waterman, 1981, Adv. Appl. Math. 2: 482, Needleman and Wunsch, 1970, J. Mol. Biol. 48: 443, Pearson and Lipman, 1988, Proc. Natl. Acad. Sci. USA 85: 2444, and Current Protocols in Molecular Biology, Ausubel et al., 1995 Suppl.).

[0029] One example of a useful algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments to show relationship and percent sequence identity, and can be obtained from the GCG sequence analysis software package, e.g., version 7.0 (Devereaux et al., 1984, Nuc. Acids Res. 12: 387-395). Another example of an algorithm that is suitable for determining percent sequence identity is the BLAST or BLAST 2.0 algorithm described in Altschul et al., 1977, Nuc. Acids Res. 25: 3389-3402 (1977) and Altschul et al., 1990, J.

Mol. Biol. 215: 403-410, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/).

[0030] Also included in the present invention are antibodies that selectively bind to the variant G_q alpha proteins described herein, but not to the corresponding native G_q alpha protein. Such antibodies include whole, chimeric, humanized, tetramer, single chain, domain-deleted and other recombinant antibodies of any immunglobulin class, as well as antibody fragments, Fv, Fab', $(Fab)'_2$, etc. Preparation of such antibodies may be performed using any method known in the art (see, e.g., Kohler and Milstein, 1975, Nature 256: 495-97; Kozbar et al., 1983, Immunology Today 4: 72; Cole et al., pp. 77-96 in Monoclonal Antibodies and Cancer Therapy, 1985). Mice or other animals may be immunized with the G_q protein variants of the invention in order to generate antibodies, which may be screened to identify those specific for the G_q variants of the invention which also do not recognize the corresponding native G_q protein.

[0031] The present invention also encompasses expression vectors comprising the nucleic acid sequences of the present invention operably linked to a promoter that functions in mammalian cells or *Xenopus* oocytes. A "promoter" is defined as an array of nucleic acid control sequences that direct transcription of a nucleic acid. A "promoter" includes all the necessary sequences near the start site of transcription, i.e., including a polymerase binding site. A promoter optionally includes distal enhancer or repressor elements which can be located as much as thousands base pairs away from the start site of transcription. Promoters may be either constitutive, i.e. active under most environmental and developmental conditions, or inducible, i.e., under specific environmental or developmental control. The term "operably linked" refers to a functional linkage between a nucleic acid expression control sequence (such as a promoter) and a second nucleic acid sequence, such as one encoding a variant G_q protein as described in the present invention, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence. An "expression vector" is a

nucleic acid construct comprising a coding nucleic acid sequence according to the invention operably linked to a promoter, which allows for recombinant production of the variant Gq proteins described herein. Expression vectors encompassed by the invention can be either incorporated into the genome of a host cell after transfection, or replicate extrachromosomally. Expression vectors can be either plasmids, viruses or nucleic acid fragments. Alternatively, coding sequences can be incorporated into the genome behind a native promoter, thereby creating an operable expression linkage following transfection. Host cells transfected with the expression vectors of the invention are also encompassed.

[0032] The present invention also includes methods for identifying a compound that modulates sensory signaling in sensory cells, the method comprising the steps of: (1) contacting the compound with a cell expressing the G_q variant protein according to claim 1; and (2) determining the functional effect of said compound upon the G_q protein variant. Typically, a cell expressing said G_q variant protein is a transfected sensory cell, or other transfected cell suitable for making functional measurements of G protein activity, i.e., *Xenopus* oocyte. Functional effects of possible modulatory compounds may be determined by measuring changes in intracellular IP3 or Ca^{2+}. Functional effects may also be determined by measuring changes in the electrical activity of the cells expressing said G_q variant protein or by observing modification of an intracellular effector enzyme. Possible modulatory compounds include agonists, antagonists, antibodies, small molecules and proteins.

[0034] Also included in the invention are methods for identifying a compound that interacts with the G_q variant protein of claim 1, comprising the steps of (1) contacting said G_q variant protein with a test compound; and (2) detecting a binding interaction between said compound and said Gq protein variant. Methods of detecting the binding of G_q protein variants to compounds can be performed wherein said G_q variant protein is linked to solid phase, either covalently or noncovalently.

[0035] The present invention also includes an artificial array of GPCRs functionally coupled to the G_q variant of claim 1, wherein said array is a model

of a native arrangement of GPCRs. For instance, the native arrangement can be an arrangement of olfactory receptors (ORs) typically seen in a mammalian nose, or an arrangement of taste receptors typically seen on a mammalian tongue. Said taste receptors typically include at least one type of taste receptor selected from the group consisting of bitter, sweet, salty, unami and sour taste receptors, in light of the observations that such taste receptors are typically arranged in spatially organized manner. The artificial arrays of the present invention are useful for analyzing the response to different sensory compounds in relation to brain activity. Such arrays will be improved by the promiscuous variant G_q proteins of the present invention, which will simplify interpretation of results that might normally be complicated by the requirement for different G protein subunits for every GPCR in such an array.

[0036] It is also envisioned that the G_q protein variants of the invention could be used in other types of functional assays such as biochemical binding assays, enzymatic assays, other cell-based assay, as well as with in vivo systems such as transgenic mice.

[0037] The following examples serve merely to illustrate the invention, and should not be construed as limiting the scope of the invention in any way.

EXAMPLES

[0038] Initially, the $G\alpha_q$ variant protein termed mGq(ON-HVD-HA) of Seq ID#5, described in Kostenis et al. (1998), was shown to functionally couple to taste receptor mT2R5 and olfactory OR I7. Previously, it had not been known whether this protein would allow functional expression of chemosensory receptors such as taste and olfactory receptors. The response using the Kostenis et al G protein was weak. Therefore, in order to improve functional coupling a series of variants was created. The variants were chimeras between the variants of the Kostenis G protein which contained the C terminal sequences from $G\alpha_{olf}$ or transducin. Variants containing the C-terminal changes exhibited improved function. The use of C-terminal replacements in G proteins had previously been reported by Conklin (Conklin et al., 1993; Conklin

et al., 1996; Coward et al., 1999) but the sequences from $G\alpha_{olf}$ or transducin had not previously been shown to function with taste or olfactory receptors.

[0039] A series of $G\alpha_q$ protein variants having the sequences listed in Fig. 3 were constructed and tested in mammalian cell-based systems and in *Xenopus* oocytes for functional coupling efficiency with bitter receptor mT2R5 and mouse olfactory receptor I7. As shown in Table I below, one set of G proteins function with a taste receptor and another set of G proteins function with an olfactory receptor. All active G proteins consisted of mouse sequences, however given the similarities between human and mouse $G\alpha_q$ proteins, it is anticipated that the human variants will also functionally couple with human chemosensory receptors.

Table I: Functional Activity of Gq Variants

Gq Variants	**Seq ID #**	**Functional Activity With Taste Receptor MT2R5**	**Functional Activity with Olfactory Receptor ml7**
MGq	1	-	N/A
MGq(ΔN)	2	-	N/A
MGq(HA)	3	-	N/A
MGq(ΔN-HA)	4	-	N/A
MGq(ΔN-HVD-HA)	5	+	+
MGq(ΔN-HVD-HA)-t5	6	++	N/A
MGq(ΔN-HVD-HA)-t44	7	++	N/A
MGq(ΔN-HV-HA)	8	-	N/A
MGq(HV-HA)	9	-	N/A
MGq(D-HA)	10	+	N/A
MGq(HVD-HA)	11	+	N/A
MGq(HV-HA)-t5	12	+	N/A
MGq(HVD-HA)-t5	13	++	N/A
MGq(ΔN-HVD-HA)-olf5	14	N/A	++
HGq	15	-	N/A
HGq(ΔN)	16	-	N/A

+ means functionally couples with chemosensory receptor
++ means functionally couples with chemosensory receptor
- means does not functionally couple
N/A mean not tested

Materials and Methods

[0040] The Ga15 chimeras were generated by PCR with mutagenic 3' primers. The sequence of our Ga15 clone corresponds to databank sequences (e.g., accession BC005439) except for a silent single nucleotide polymorphism shown in bold underline below. The last six codons of Ga15 and the sequences they were replaced with are shown in italic underline below. The Ga15 chimeras were generated with 5' AscI sites (GGCGCGCCgcc joined to the start ATG) and 3' NotI sites (GCGGCCGC joined to the stop TGA) and cloned as AscI-NotI fragments in the AscI-NotI polylinker sites of the pEAK10 expression vector (Edge Biosystems).

Ga 15 (SEQ ID NO:27)

Atggcccggtccctgacttggggctgctgtccctggtgcctgacagaggaggagaagactgccgccagaat
cgaccaggagatcaacaggattttgttggaacagaaaaaacaagagcgcgaggaattgaaactcctgctgt
tggggcctggtgagagcgggaagagtacgttcatcaagcagatgcgcatcattcacggtgtgggctactcgg
aggaggaccgcagagccttccggctgctcatctaccagaacatcttcgtctccatgcaggccatgatagatgc
gatggaccggctgcagatccccttcagcaggcctgacagcaagcagcacgccagcctagtgatgacccag
gacccctataaagtgagcacattcgagaagccatatgcagtggccatgcagtacctgtggcgggacgcggg
catccgtgcatgctacgagcgaaggcgtgaattccaccttctggactccgcggtgtattacctgtcacacctgg
agcgcatatcagaggacagctacatccccactgcgcaagacgtgctgcgcagtcgcatgcccaccacagg
catcaatgagtactgcttctccgtgaagaaaaccaaactgcgcatcgtggatgttggtggccagaggtcaga
gcgtaggaaatggattcactgttttgagaacgtgattgccctcatctacctggcctccctgagcgagtatgacca
gtgcctagaggagaacgatcaggagaaccgcatggaggagagtctcgctctgttcagcacgatcctagagc
tgccctggttcaagagcacctcggtcatcctcttcctcaacaagacggacatcctggaagataagattcacac
ctcccacctggccacatacttccccagcttccagggaccccggcgagacgcagaggccgccaagagcttc
atcttggacatgtatgcgcgcgtgtacgcgagctgcgcagagccccaggacggtggcaggaaaggctccc
gcgcgcgccgcttcttcgcacacttcacctgtgccacggacacgcaaagcgtccgcagcgtgttcaaggacg
tgcgggactcggtgctggcccggtacctggacgagatcaacctgctgtga

GACTGTGGCCTCTTCTGA Gai1 (SEQ ID NO:28)
GAGTACAATCTGGTCTGA Gaq (SEQ ID NO:29)
CAGTATGAGCTCTTGTGA Gas (SEQ ID NO:30)
GAGTGCGGCCTCTACTGA Gai3 (SEQ ID NO:31)
GGATGCGGACTCTACTGA Gao (SEQ ID NO:32)
TACATCGGCCTCTGCTGA Gaz (SEQ ID NO:33)
GACATCATGCTCCAATGA Ga12 (SEQ ID NO:34)
CAACTAATGCTCCAATGA Ga13 (SEQ ID NO:35)
CACCAGGTTGAACTCTGA Ga14 (SEQ ID NO:36)

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